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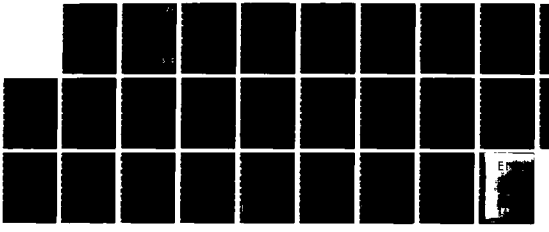

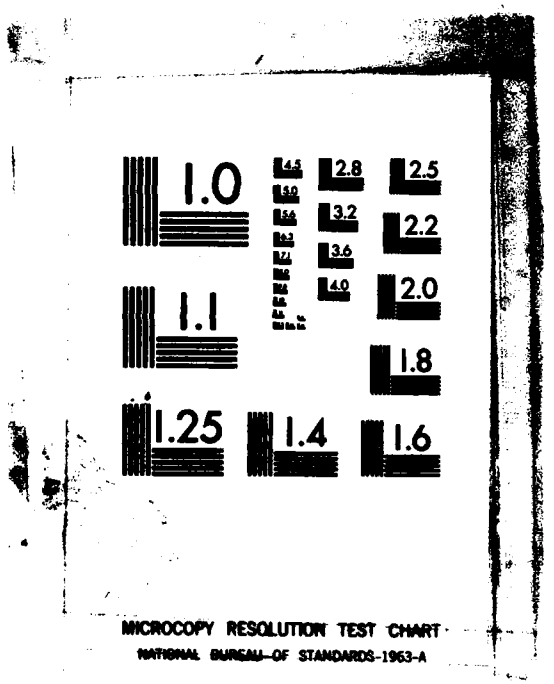
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MEAN PLATELET VOLUME AND PLATELET IMMUNOFLUORESCENCE AS INDICATORS OF PLATELET COMPATIBILITY

by

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In the study reported here, two methods, one new and one a modified version of a previously used method, were employed for the evaluation of platelet compatibility. Platelet compatibility tests were performed on fresh, liquid-stored and washed previously frozen platelets. The immunofluorescent techniques of platelet antibody identification used in this study have been described previously. The Coulter fluorescence activated cell sorter was used to measure alloantibodies and platelet specific antibodies (anti-PLA). The Coulter Counter H4 channelyzer was used to measure mean platelet volume (MPV).

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Treatment of normal donor platelets with patient sera containing alloantibodies produced an increase in MPV, unlike treatment of the same donor platelets with autologous serum. An increase in MPV also was seen after treatment of blood group A platelets with serum from blood group O donors, but none was seen after treatment with other mismatched ABO groups.

ABSTRACT

In the study reported here, two methods, one new and one a modified version of a previously used method, were employed for the evaluation of platelet compatibility. Platelet compatibility tests were performed on fresh, liquid-stored and washed previously frozen platelets. The immunofluorescent techniques of platelet antibody identification used in this study have been described previously. The Coulter fluorescence activated cell sorter was used to measure alloantibodies and platelet specific antibodies (anti-PLA). The Coulter Counter H4 channelyzer was used to measure mean platelet volume (MPV).

Treatment of normal donor platelets with patient sera containing alloantibodies produced an increase in MPV, unlike treatment of the same donor platelets with autologous serum. An increase in MPV also was seen after treatment of blood group A platelets with serum from blood group O donors, but none was seen after treatment with other mismatched ABO groups.

INTRODUCTION

Approximately 50% of all patients who receive repeated platelet transfusions become alloimmunized to random donor platelets;[5] about 75% of those affected will respond to treatment with HLA-matched platelets, and such treatment requires the availability of a very large pool of HLA-typed donor platelets.

What is needed is a simple platelet crossmatching test that could identify suitable donors for alloimmunized patients. Although a number of methods have been tested (e.g., lymphocytotoxicity, leukoagglutination, platelet aggregation, radioimmunoassays, platelet immunofluorescence, and an enzyme-linked immunoabsorbant assay),[2,3,7,10] results have been inconsistent.

We report here on two methods used in our laboratory to demonstrate alloantibodies in thrombocytopenic patient sera. With one method the Coulter H4 channelyzer was used to study changes in platelet size in the presence of platelet alloantibodies, and in the other the Coulter fluorescence-activated cell sorter was used to measure platelet immunofluorescence in the presence of alloantibodies.

MATERIALS AND METHODS

Platelet Preparation

After obtaining informed consent from each normal volunteer, a 40 ml volume of blood was collected from each into a plastic syringe for compatibility testing on fresh donor platelets. Ten ml was placed in a glass tube and allowed to clot for 1 hour prior to centrifugation to obtain the serum. The remaining 30 ml of blood was divided into four 17 X 100 mm polypropylene tubes, each containing 2.5 ml of 1.2% EDTA in 0.9% NaCl. The platelet-rich plasma (PRP) was isolated by centrifugation at 280 X g for 5 minutes.

The PRP from all the blood samples was pooled, and the platelets were concentrated by centrifugation at 1000 X g for 10 minutes. The platelet-poor plasma (PPP) was removed and the platelet pellet was resuspended in 2 ml of 0.01 M Tyrodes-EDTA buffer (Appendix I). The resuspended platelets were washed by dilution with 8 ml of buffer, the platelets were concentrated by centrifugation at 1000 X g for 5 minutes, washed twice more, and the final platelet pellet resuspended in 1.5 ml of the Tyrodes-EDTA buffer. The platelet concentration was measured and adjusted to 200,000/mm^3 by addition of buffer.

The liquid-stored donor platelets used for the platelet compatibility test had been stored at 22 ± 2 C for 24 hours in a PL-146 or PL-732 transfer pack (Fenwal Laboratories, Deerfield, IL) held on a lateral Eberbach shaker oscillating at 70 cycles per minute. A 5.0 ml aliquot of these platelets

was diluted with 0.01 M Tyrodes-EDTA buffer, washed three times as previously described, and after the third wash resuspended in 0.01 M Tyrodes-EDTA buffer at a concentration of 200,000/mm^3.

The previously frozen donor platelets used for compatibility testing had been frozen with dimethylsulfoxide (DMSO) and washed as previously described.[9] A 5 ml aliquot of the washed previously frozen platelets was diluted with 0.01 M Tyrodes-EDTA buffer, washed three times in this buffer as previously described, and after the third wash resuspended in 0.01 M Tyrodes-EDTA buffer at a concentration of 200,000/mm^3.

Sera

For each experiment, autologous serum from the platelet donor was used as the control. In addition, serum samples were obtained from normal ABO incompatible donors: three patients with posttransfusion purpura with anti-$P1^A$ antibodies, and three alloimmunized thrombocytopenic patients.

Platelet Treatment

The donor platelets (fresh, liquid-stored and previously frozen) after washing and resuspension in 0.01 M Tyrodes-EDTA buffer, were divided into 0.2 ml aliquots in 5 ml polypropylene tubes. The platelets were diluted with either 0.2 ml of autologous donor serum or 0.2 ml of patient sera and incubated in a 37 C water bath for 30 minutes. In some experiments, the platelets were incubated with 0.2 ml or 0.4 ml of sera.

Following incubation with the sera, the platelets were washed again by dilution with 4.0 ml of Tyrodes-EDTA buffer. The platelets were centrifuged at 1000 X g for 5 minutes and then washed twice more, resuspended in 0.2 ml of buffer, and the platelet volume distribution recorded.

Fluorescent Antibody Labeling

For fluorescent antibody testing the donor platelets were washed with Tyrodes-EDTA buffer, incubated with sera, and washed again in Tyrodes-EDTA buffer. The platelets were diluted with 0.2 ml of goat anti-human fluoresceinated IgG($F(ab')_2$)fraction (Cappel Laboratories, Cochranville, PA) that had been diluted 1:20 with phosphate-buffered saline. In other experiments, 1:10 and 1:40 dilutions were also tested. The platelets were incubated for 30 minutes at room temperature and then washed three times with Tyrodes-EDTA buffer. The platelet pellets were resuspended in 0.2 ml of buffer, and immunofluorescence was measured with the Coulter fluorescence activated flow cytometer. In some experiments, fresh samples and samples after fixation and storage in a 1% formalin solution were analyzed to determine whether fixation altered the data obtained from the fluorescence activated cell sorter.

Platelet Volume Measurement

After incubation and washing, the platelets were diluted with phosphate-buffered saline that was filtered through a 0.22 micron filter and dilutions of 1:10,000 were tested. The settings for the Coulter H4 channelyzer were: Gain 2.5, Amplification 0.5, Aperture Current 0.5, Lower Threshhold 6 and Upper Threshhold 80. The platelets were sized with a 50 micron aperture using the expanded logarithmic mode. The instrument was calibrated with latex particles (2.02 u diameter) and the interval for mean platelet volume assessment was set between 4 and 45 cubic microns. In one experiment, platelet volume was measured with both the Coulter H4 channelyzer and the Coulter S+ instrument.

Fluorescent Data Analysis

Data collected with the Coulter cell sorter were analyzed with the EASY I extended analysis system using the immune program. The fluorescence of the platelets incubated with the patient's serum was corrected for the fluorescence of the negative control and the percentage of fluorescent-positive cells was determined.

RESULTS

An unexpected observation was that an increase in mean platelet volume of donor platelets occurred when they were incubated with thrombocytopenic patient serum (Figure 1).

Studies were performed to determine if the increase in MPV was due to the presence of alloantibodies or if it was due to ABO isoagglutinins anti-A and anti-B. Platelets from normal donors with A, B, and O blood group types were treated with autologous serum and serum from normal volunteers with other blood group types (Tables 1A and 1B). Sera from donors with blood group type O containing anti-A and anti-B isoagglutinins always caused type A platelets to swell (range 7-24%) (Table 1A). Mixtures of B and O platelets with sera containing anti-A and anti-B isoagglutinins demonstrated no change or a slight decrease in MPV.

Platelets from normal volunteers with blood group types A and O were tested with serum from an alloimmunized patient having blood group type O-positive (Table 2). Type A platelets treated with the patient's serum containing anti-A isoagglutinin demonstrated an increase in MPV ranging from 9.7 to 48.9%. Type O platelets incubated with the patient's O serum also demonstrated an increase in MPV ranging from 1.0 to 12.7%. The MPV did not increase when donor platelets were incubated with increasing volumes of autologous serum but the MPV did increase when donor platelets were incubated with increasing volumes of incompatible sera (Table 2).

Platelets from normal volunteers with blood group types A, B and O

were incubated with serum from an alloimmunized patient with blood group type A-positive (Table 3). Increases in MPV ranged from 8.3 to 26.2% regardless of the donor's blood group type.

MPV measured in sera from three patients with posttransfusion purpura and anti-$P1^{A1}$ antibodies was compared with that in platelets from normal volunteers (Table 4). MPV was higher in normal donor platelets treated with undiluted patient serum than in normal platelets treated with autologous serum. ABO blood types of the patients were not known. Sera from patients #1 and #2 produced increases in MPV ranging from 14.8 to 90.1%. Serum from patient #3 produced only a 4.3% increase in MPV. Dilution of the patient's serum with normal saline, Tyrodes buffer, or control serum usually eliminated the increased MPV (Table 4). When donor platelets were incubated with dilutions of 3 parts patient serum and 1 part diluent, the increase in MPV was similar to that seen when donor platelets were incubated with undiluted patient serum. When the serum was diluted more than 3:1, usually there was no change in MPV, or MPV was lower than that seen with control serum (Table 4).

The sera containing anti-$P1^{A1}$ antibodies were also tested against normal platelets using immunofluorescence as measured with the Coulter fluorescence activated cell sorter (Table 5). A 1:20 dilution of the goat anti-human IgG (F(ab')2) produced the best results. Sera from all three patients resulted in a marked increase in immunofluorescence in comparison to the controls (Table 5). Dilution of the patient sera did not result in a loss of ability to detect the antibodies. Dilution of serum from patient #2 did result in a progressive decrease in immuno-

fluorescence, suggesting that this patient had a low antibody titer. Sera from patients #1 and #3 demonstrated no loss of immunofluorescence at dilutions of 1 to 3.

An attempt was made to predict the response of an alloimmunized patient to ABO compatible platelet transfusions, using changes in mean platelet volume and immunofluorescence (Table 6). The patient's sera caused an increase in MPV and markedly positive immunofluorescence when tested against platelets from two ABO compatible donors. Illustrations of changes in MPV and immunofluorescence are shown in Figures 2 and 3. The patient was given a transfusion of platelets from each of the ABO compatible donors and the platelet count did not increase after the transfusions. An increase in MPV and increased immunofluorescence were observed when fresh, liquid-stored or previously frozen platelets were used for the compatibility tests (Table 6).

Some of the immunofluorescent samples were analyzed fresh and then fixed with 1% formalin. Analyses of the fixed samples over the next two weeks showed results identical to those seen with the fresh samples.

The Coulter S+ instrument was used to measure changes in MPV that occurred with alloantibodies. Platelets from a normal volunteer were treated with autologous serum or with serum from an alloimmunized patient, after which the platelets were sized with both the Coulter H4 and the S+ instrument (Table 7). The MPV and changes in MPV were similar with both instruments.

DISCUSSION

Simple platelet crossmatch tests are needed to screen possible donors of platelets for alloimmunized patients. Currently, available methods are time-consuming and have produced variable results. In this report, we have described our experience with a new method of platelet antibody testing and an advancement in the measurement of the previously described immunofluorescent technique.[2,10]

Our data demonstrated that normal donor platelets incubated with patient sera containing alloantibodies have larger MPV than the same donor platelets incubated with autologous serum. Changes in MPV were detected with the Coulter H4 channelyzer; an increase in MPV was associated with no change in platelet counts following transfusion. The test was easy to do and less time-consuming than currently available tests.

Blood group O serum containing anti-A and anti-B isoagglutinins always caused an increase in the MPV of blood group A platelets, and studies are in progress to determine whether the increase in MPV represents isoagglutinins alone, alloantibodies alone, or a combination of these antibodies. Blood group B serum, which contains only anti-A isoagglutinins, did not cause an increase in the MPV of group A platelets. Type O serum which contains anti-A and anti-B isoagglutinins and type A serum which contains only anti-B isoagglutinins did not cause an increase in the MPV of blood group B platelets.

The significance of ABO compatibility in platelet transfusion therapy is a controversial subject.[1,4,6,8] Aster and his colleagues reported that

patients with blood group type O demonstrated poor posttransfusion platelet increments when they were transfused platelets from donors with blood group type A1 or A_1B, but that these same patients had excellent posttransfusion increments when they received platelets from a group B donor.[1] The explanation given for this was that blood group O donors have higher titers of anti-A isoagglutinins than anti-B isoagglutinins, a clinical observation supported by our observation that treatment with O serum in vitro caused A platelets to swell, whereas other ABO mismatches produced no increase in MPV. Duquesnoy and co-workers[4] have reported a significantly reduced platelet recovery when ABO incompatible HLA-matched platelets were given instead of ABO compatible HLA-matched platelets. Lohrmann[8] reported that ABO incompatibility of HLA-compatible platelet donors did not affect the posttransfusion platelet increments; eleven patients received type B incompatible platelets and eight patients received type A incompatible platelets. There was no indication whether the recipients in Lohrmann's study were type B or type O. Freireich and associates[6] reported that a patient who received multiple platelet transfusions from donors of different blood types exhibited no adverse effect on posttransfusion platelet increment. In this study a group O patient was given 6 platelet transfusions from a group B donor, 3 platelet transfusions from a group O donor, and 4 platelet transfusions from a group A donor, with similar increases in posttransfusion platelet counts. Neither the number of platelets transfused nor the percent platelet recovery was reported, nor was it reported whether the group A donor was A_1 or A_2. A controlled study is needed to determine if group O patients can be given group A platelets without

adversely influencing platelet posttransfusion survival. Type specific platelets should be used when possible.

The reason for the increase in MPV after exposure to alloantibodies or to isoagglutinins is not known. It is not due to antibody coating alone since platelets treated with diluted anti-Pl^{A1} antibody are coated with antibody detected by immunofluorescence and antibody coated platelets did not have an increase in MPV. The swelling may be due to complement fixation and studies are needed to evaluate this possible mechanism.

Immunofluorescence is a sensitive method of demonstrating anti-platelet antibodies. This method requires 5 to 6 hours of cell preparation and the availability of a fluorescence activated cell sorter which limits the clinical usefulness of this method.

Liquid-stored and frozen washed platelets can be used to perform the platelet compatibility testing. Liquid-stored platelets available in the blood bank can be evaluated prior to transfusion. Today, panels of potential platelet donors can be frozen with DMSO, and the panels can be tested to find the best possible donor for alloimmunized thrombocytopenic patients.

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TABLE 1A

THE EFFECT OF ABO ISOAGGLUTININS ON MEAN PLATELET VOLUME AS MEASURED WITH THE COULTER H4 CHANNELYZER

Experiment #	Platelet Donor ABO Type	Test Serum ABO Type	Mean Platelet Volume (u^3)	% Change MPV of Control
1	A	A (auto)	4.57	-----
	A	A (auto)	4.57	-----
	A	O	4.90	+7.2%
	A	O	4.90	+7.2%
	O	O (auto)	4.27	-----
	O	O (auto)	4.27	-----
	O	A	4.27	-----
	O	A	4.27	-----
2	A	A (auto)	7.59	-----
	A	B	7.24	-4.6%
	A	O	8.81	+16.0%
3	A	A (auto)	15.50	-----
	A	B	15.50	-----
	A	O	17.89	+15.0%
	B	B (auto)	17.89	-----
	B	A	16.59	-7.3%
	B	O	17.89	-----
4	A	A (auto)	18.56	-----
	A	B	18.25	-1.7%
	A	O	23.03	+24.1%
	B	B (auto)	19.90	-----
	B	A	19.90	-----
	B	O	17.90	-10.0%
5	O	O (auto)	15.49	-----
	O	A	15.00	-3.2%
	O	B	14.50	-6.4%
6	O	O (auto)	20.70	-----
	O	A	19.25	-7.0%
	O	B	20.00	-3.4%

TABLE 1B

THE EFFECT OF ABO ISOAGGLUTININS ON MEAN PLATELET VOLUME

Platelet Donor ABO Type	Test Serum ABO Type	Effect	Range (% Change)	n
A	O	Swelling	+7.2 to +24.1%	5
B	O	No change or shrinking	-10.0% to 0	2
A	B	No change or shrinking	-4.6% to 0	3
B	A	No change or shrinking	-7.3% to 0	2
O	A	No change or shrinking	-7.0% to 0	4
O	B	Slight shrinking	-3.4% to -6.4%	2

TABLE 2

MEAN PLATELET VOLUME OF A+ OR O+ DONOR PLATELETS TREATED WITH EITHER AUTOLOGOUS SERUM OR SERUM FROM AN ALLOIMMUNIZED THROMBOCYTOPENIC O+ PATIENT*

Donor Platelets	Test Serum	Mean Platelet Volume (u^3)	% Change of MPV of Control
A+, #1	Autologous	7.52	----
	Patient	11.20	+48.9%
A+, #1	Autologous	5.31	----
	Patient	6.23	+17.0%
A+, #1	0.2 ml Autologous	5.32	----
	0.4 ml Autologous	5.32	----
	0.2 ml Patient	6.55	+23.1%
	0.4 ml Patient	6.40	+20.3%
A+, #1	0.4 ml Autologous	5.14	----
	0.4 ml Patient	5.64	+9.7%
O+, #1	0.2 ml Autologous	3.84	----
	0.4 ml Autologous	3.84	----
	0.2 ml Patient	3.88	+1.0%
	0.4 ml Patient	4.07	+5.9%
O+, #1	0.2 ml Autologous	4.23	----
	0.4 ml Autologous	4.23	----
	0.2 ml Patient	4.28	+1.2%
	0.4 ml Patient	4.38	+3.5%
O+, #2	Autologous	10.30	----
	Patient	11.61	+12.7%

*Patient was refractory to all platelet transfusions

TABLE 3

MEAN PLATELET VOLUME OF A, B, OR O DONOR PLATELETS TREATED WITH AUTOLOGOUS SERUM OR SERUM FROM AN ALLOIMMUNIZED A+ PATIENT*

Donor Platelets	Test Serum	Mean Platelet Volume (u^3)	% Change of MPV of Control
A+	Autologous	18.67	----
	A+ Patient	21.00	+12.5%
A+	Autologous	12.00	----
	A+ Patient	13.00	+8.3%
B+	Autologous	7.85	----
	A+ Patient	8.50	+8.3%
B+	Autologous	22.20	----
	A+ Patient	27.50	+23.9%
O+	Autologous	10.30	----
	A+ Patient	13.00	+26.2%

*The patient had received multiple blood transfusions and a cadaver kidney transplant

TABLE 4

THE EFFECT OF $P1^{A1}$ ANTISERUM ON MEAN PLATELET VOLUME OF NORMAL VOLUNTEER PLATELETS AS MEASURED WITH THE COULTER H4 CHANNELYZER

Experiment #	Test Serum	Mean Platelet Volume (u^3)	% Change of MPV of Control
1	Autologous	8.30	-----
	PLA #1	9.75	+17.5%
	PLA #1	9.53	+14.8%
2	Autologous	6.15	-----
	Autologous	6.44	-----
	PLA #1	9.53	+51.3%
	PLA #1 (diluted 1:1 NS)	5.74	-8.9%
	PLA #1 (diluted 1:3 NS)	6.44	-----
3	Autologous	6.33	-----
	PLA #1	Lysed Control Platelets	
4	Autologous	7.10	-----
	PLA #1	13.50	+90.1%
	PLA #1 (diluted 3:1 Tyrodes)	16.09	+126.6%
	PLA #1 (diluted 1:1 Tyrodes)	6.48	-8.7%
	PLA #1 (diluted 1:3 Tyrodes)	6.48	-8.7%
5	Autologous	8.22	-----
	PLA #1	10.66	+29.7%
	PLA #1 (diluted 3:1 Tyrodes)	10.10	+22.9%
	PLA #1 (diluted 1:1 Tyrodes)	7.85	-4.5%
6	Autologous	9.51	-----
	PLA #1	11.97	+25.9%
	PLA #1 (diluted 3:1 Tyrodes)	9.29	-2.3%
7	Autologous	17.00	-----
	PLA #1	21.50	+26.5%
	PLA #1 (diluted 1:3 NL serum)	14.00	-17.6%

TABLE 4 (CONTINUED)

Experiment #	Test Serum	Mean Platelet Volume (u^3)	% Change of MPV of Control
8	Autologous	28.2	-----
	PLA #2	33.1	+17.4%
	PLA #2 (diluted 1:1 with NL serum)	28.8	+2.1%
	PLA #2 (diluted 1:3 with NL serum)	28.2	-----
9	Autologous	18.5	-----
	PLA #3	19.3	+4.3%
	PLA #3 (diluted 1:1 with NL serum)	18.5	-----
	PLA #3 (diluted 1:3 with NL serum)	18.5	-----
10	Autologous	17.2	-----
	PLA #2	22.2	+29.1%
11	Autologous	16.1	-----
	PLA #2	25.0	+55.3%

NL = Normal

TABLE 5

THE EFFECT OF $P1^{A1}$ ANTISERUM ON PLATELET IMMUNOFLUORESCENCE AS MEASURED WITH GOAT FITC ANTI-HUMAN IgG(F(ab')2)ASSESSED WITH THE COULTER FLUORESCENCE ACTIVATED CELL SORTER

Experiment #	Test Serum	FITC Dilution	% Positive Cells in Relation to Control
1	PLA #1	1:20	97%
	PLA #1	1:40	30%
2	PLA #1	1:10	60%
	PLA #1	1:20	70%
3	PLA #1	1:20	95%
	PLA #1 (diluted 1:1 with normal serum)	1:20	94%
	PLA #1 (diluted 1:3 with normal serum)	1:20	98%
4	PLA #2	1:20	52%
	PLA #2 (diluted 1:1 with normal serum)	1:20	28%
	PLA #2 (diluted 1:3 with normal serum)	1:20	15%
5	PLA #3	1:20	98%
	PLA #3 (diluted 1:1 with normal serum)	1:20	97%
	PLA #3 (diluted 1:3 with normal serum)	1:20	97%

TABLE 6

THE EFFECT ON MEAN PLATELET VOLUME AND IMMUNOFLUORESCENCE WHEN FRESH, LIQUID-STORED, OR PREVIOUSLY FROZEN ABO COMPATIBLE DONOR PLATELETS ARE TREATED WITH SERUM FROM AN ALLOIMMUNIZED PATIENT*

Donor #/ Platelet Preparation	Test Serum	Mean Platelet Volume (u^3)	% Change of MPV of Control	% Fluorescent Cells in Relation To Control
Donor #1/ Stored at 22 C for 24 hours	Autologous	11.30	----	----
	Patient	11.96	+5.8%	70.0%
Donor #2/ Fresh	Autologous	12.50	----	----
	Patient	13.90	+11.2%	61.2%
Stored at 22 C for 24 hours	Autologous	13.10	----	----
	Patient	15.60	+18.8%	59.9%
Previously frozen in 6% DMSO	Autologous	14.60	----	----
	Patient	16.40	+12.2%	74.2%

*The patient received platelet transfusions from both donors without an increment in her platelet count

TABLE 7

THE EFFECT ON MEAN PLATELET VOLUME OF NORMAL DONOR PLATELETS TREATED WITH EITHER AUTOLOGOUS SERUM OR SERUM FROM AN ALLOIMMUNIZED THROMBOCYTOPENIC PATIENT* AS MEASURED WITH THE COULTER H4 CHANNELYZER AND THE COULTER S+

	COULTER H4		COULTER S+	
Test Serum	Mean Platelet Volume (u^3)	% Change of MPV of Control	Mean Platelet Volume (u^3)	% Change of MPV of Control
Autologous	12.20	-----	11.10	-----
Patient	13.76	+12.8%	12.70	+14.4%

*Patient was refractory to all platelet transfusions, including transfusions from her siblings

FIGURE 1

Platelet volume distribution pattern of normal donor platelets treated with autologous serum or serum from an alloimmunized thrombocytopenic patient.

FIGURE 1

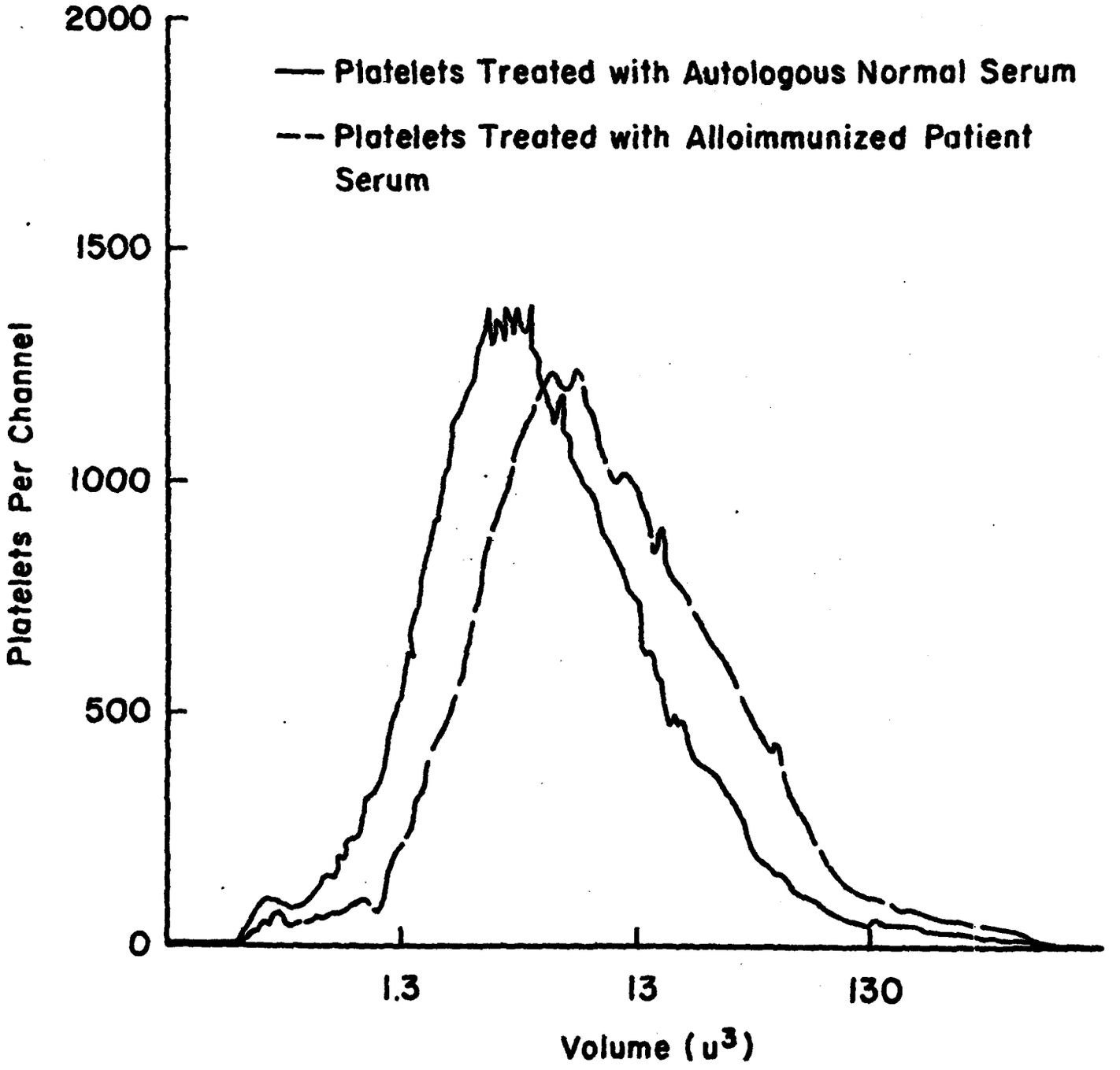

2000
1500
1000
500
0
Platelets Per Channel
Platelets Treated with Autologous Normal Serum
Platelets Treated with Alloimmunized Patient Serum
1.3
13
130
Volume (u3)

FIGURE 2

Platelet volume distribution pattern of ABO compatible platelets treated with autologous serum or serum from an alloimmunized thrombocytopenic patient.

FIGURE 2

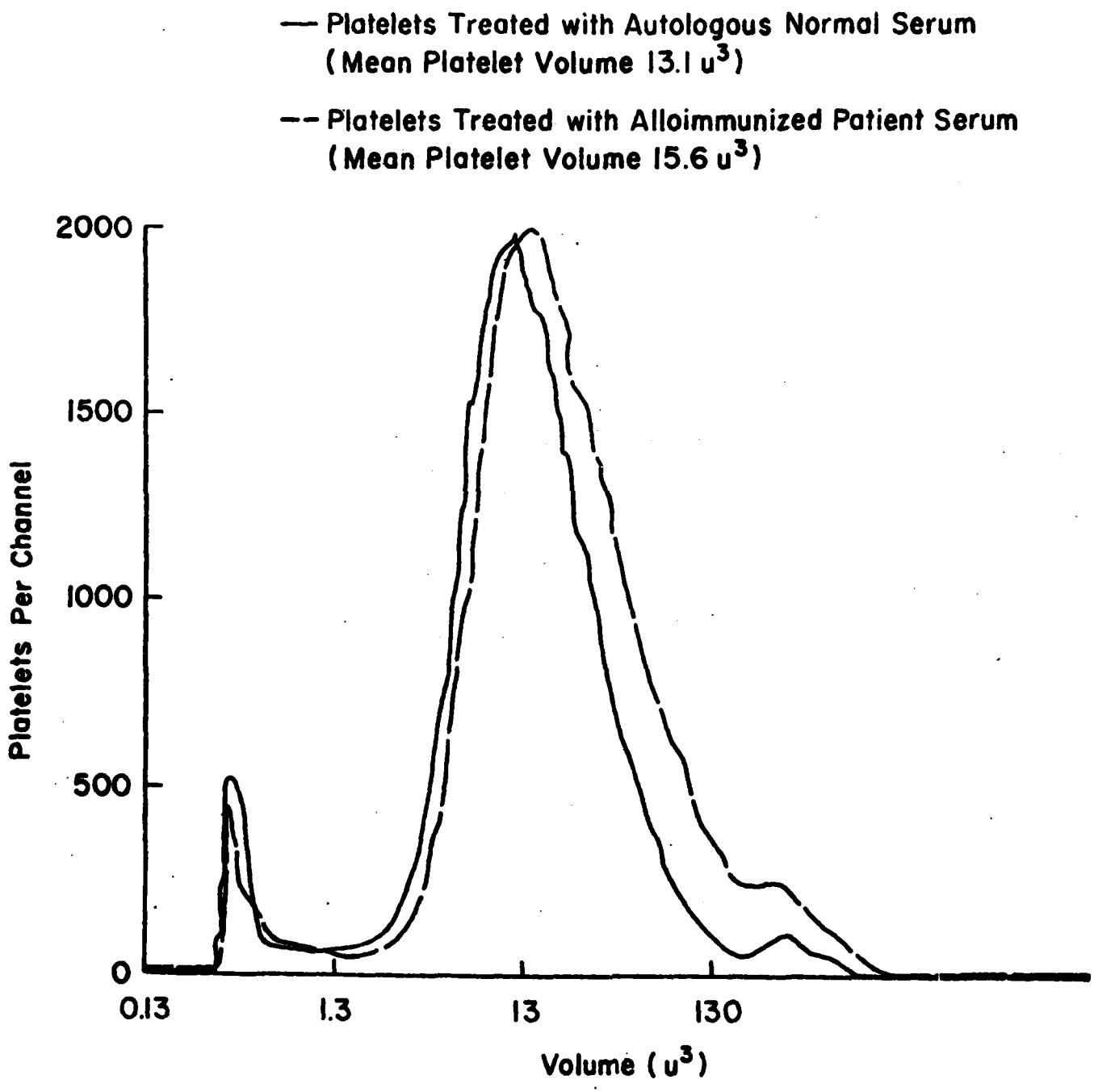
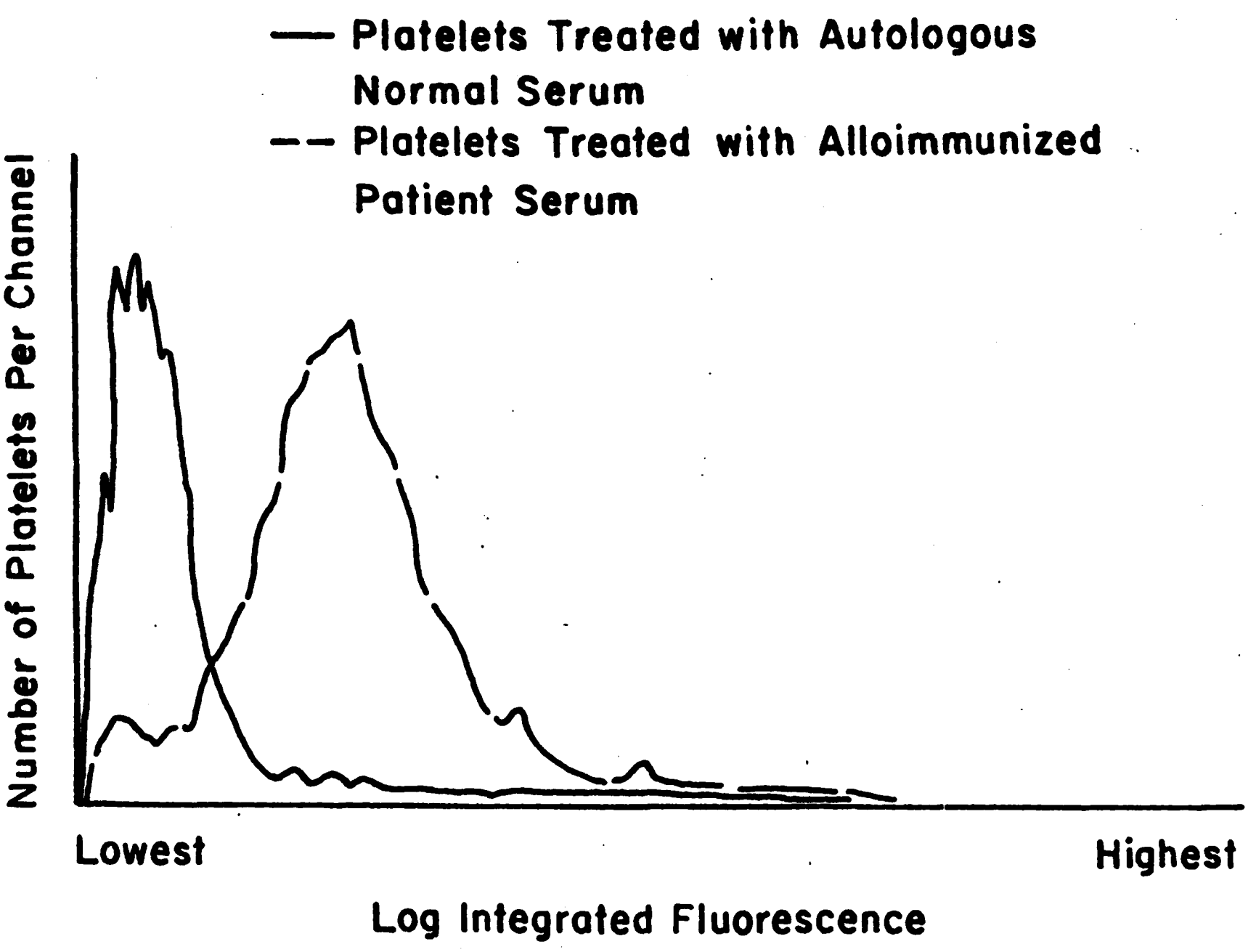

— Platelets Treated with Autologous Normal Serum
(Mean Platelet Volume 13.1 u³)
-- Platelets Treated with Alloimmunized Patient Serum
(Mean Platelet Volume 15.6 u³)
2000
1500
1000
500
0
Platelets Per Channel
0.13
1.3
13
130
Volume (u³)

FIGURE 3

Immunofluorescence of ABO compatible platelets treated with autologous serum or with serum from an alloimmunized patient treated with fluoresceinated goat anti-human F(ab')2, and the samples were analyzed on the Coulter cell sorter.

FIGURE 3


— Platelets Treated with Autologous Normal Serum
-- Platelets Treated with Alloimmunized Patient Serum
Number of Platelets Per Channel
Lowest
Highest
Log Integrated Fluorescence

APPENDIX I

PREPARATION OF 0.01 M TYRODES-EDTA BUFFER

Prepare:

Tyrodes Stock I

KCL	-	0.8 g
NaCl	-	32.0 g
$NaHCO_3$	-	4.0 g
NaH_2PO_4	-	.232 g

in 200 ml distilled H_2O

0.3 N NaOH

6 g NaOH in 500 ml distilled H_2O

0.86 M EDTA stock

22.33 g Na EDTA in 500 ml distilled H_2O
This is 0.12 M EDTA with pH 4.5.
Add 3 NaOH to EDTA until pH reaches 7.2-7.6.

$$\text{Final Molarity of EDTA} = \frac{\text{starting volume of 0.12 M EDTA}}{\text{starting volume of EDTA + volume 0.3 N NaOH}} (0.12)$$

*should equal about 0.086 M EDTA

Tyrodes Stock II (Prepare Fresh)

Tyrodes Stock I	25 ml
Bovine albumin	1.75 g
Dextrose	0.50 g

QS to 500 ml with distilled H_2O

0.01 M Tyrodes-EDTA

0.086 M EDTA - 57.5 ml
QS to 500 ml with Tyrodes Stock II (Fresh)

*This should be made fresh each day. The Tyrodes Stock I, EDTA, and 0.3 N NaOH can be kept at 4 C and reused.

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